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Pre-concentration of ultra-trace furan in beverage samples and its determination by high performance liquid cromatography

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Abstract

Background: Furan has been considered to be toxic and carcinogenic. In this research, a new method was applied for the pre-concentration of furan in beverage samples followed by high-performance liquid chromatography-ultraviolet detection (HPLC-UV) determination.

Results: Determination was carried out by a UV detection at 205 nm. Some of the most important parameters, including extraction solvent, extraction time, sample temperature and volume, helium flow rate and salt effect were investigated and optimized. Linear calibration curve was obtained over a concentration range of 0.005 to 0.3 μg/mL with coefficient of determination ($R^2 = 0.998$). Limit of detection and relative standard deviation were obtained as 0.001 μg/mL and 2.21% ($n = 5$), respectively.

Conclusions: The proposed method was applied successfully for the determination of furan in beverage samples. Among them, cola had a detectable amount of furan.

Keywords: Furan, Beverage samples, Pre-concentration, HPLC-UV

Background

Chemical food safety is a topic with an increasing concern within the respective community, although due to the present risk management procedures, the acute threat posed by harmful chemicals is very uncommon. Instead, there has been much more interest focused on the minute concentrations and consequent potential chronic symptoms. It has been postulated that chemical contaminants found in foods (and feeds) might be etiological factors behind several diseases (e.g., cancer), the causes of which are still greatly unknown. In addition to the environmental and agrochemical residues as well as natural toxins, contaminants produced during the food processing or storage, such as acrylamide, polycyclic aromatic hydrocarbons (compounds), benzene and furan, are becoming an issue.

Furan (C_4H_4O), a lipophilic contaminant, is formed during heating process for the manufacture of foods. Furan is an aromatic heterocyclic compound, which is highly volatile with a boiling point of 31°C. Furan has been identified in hot-air dried, baked, fried and roasted food items, such as cereal products and coffee as well as can- or jar-prepared foods, at low concentration levels (<200 ng/g) [1]. Its presence in food is known since the 1960s as one of the Maillard reaction products formed in the thermal processes of foods such as birch syrup, caramel, coffee, bread and cooked meat [2-10]. Nowadays, the formation of furan remains unclear, although it is known that its generation is related to the thermal degradation of carbohydrates, thermal oxidation of polyunsaturated fatty acids and/or thermal decomposition of ascorbic acid or its derivatives [11-14]. In recent years, the study of furan in foods has received special attention by several international food organizations such as the US Food and Drugs Administration and the European Food Safety Authority [15-17]. Due to its toxicity and carcinogenicity, furan has been included by the

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International Agency for Research on Cancer in the Group 2B as possibly carcinogenic to humans [18-20] and has been classified by the US Department of Health and Human Services as a human pathogen [21]. Therefore, there is a need to propose reliable methods for its determination in foods in order to obtain information about its occurrence as well as the source of exposure, mechanisms of formation and toxicology. The analysis of furan in food is not easy due to its high volatility, low molecular weight and low concentration levels in food products.

The most common analytical technique used to determine the concentration of furan is headspace extraction combined with gas chromatography-mass spectrometric (GC-MS) detection [15,22,23]. Later on, solid-phase microextraction followed by GC-MS has been developed [22,24]. Both approaches are convenient sample preparation techniques as they are very simple and demand no expensive equipment for sample extractions/concentration.

In the present study, purge-and-trap method combined with HPLC was applied for the determination of the amount of furan in food samples. The detection wavelength for the absorption of furan was set at 205 nm [25]. A systematic study of the effective parameters on the extraction and desorption processes was used to obtain maximum sensitivity and selectivity. In addition, we investigated the applicability of the proposed method for the quantification of furan in solid and liquid food samples. The proposed method provides relatively low limits of detection (LOD; 0.001 μg/mL).

Methods

Experimental

Materials and instruments

Furan, water, methanol and acetonitrile of HPLC grade were purchased from Merck and Co. Inc. (Darmstadt, Germany). All Other reagents were of analytical grade and obtained from Merck.

Chromatographic conditions for sample analysis

Chromatographic Separation Were Carried out on a HPLC Instrument (Waters, Model 600, USA) equipped with a Waters 2487 UV-Vis Detector. A Zorbax Extend C18 column (250 mm × 5 mm, with 5 μm particle size) was purchased from Agilent Technologies Inc. (Wilmington, DE, USA). A mixture of water/methanol/acetonitrile (70:20:10 vol.%) at the flow rate of 1 mL/min was used as the mobile phase in the isocratic elution mode. The injection volume was 20 μL, and the detection wavelength was set at 205 nm.

Extraction procedure

A balloon containing standard solutions was connected to the trapping unit which was placed in the ice bath (Figure 1). Trapping unit was filled with glass beads to increase its trappig efficiency. From the other side of the balloon, helium gas was purged through the heated sample solution under stiring condition. The interface between the balloon and trapping unit was heated by industrial drier to prevent furan condensation and it's return to the balloon.

Results and discussion

Selection of an appropriate extraction solvent

The selection of an appropriate solvent is important to achieve the highest sensitivity. Different solvents such as dimethy formamide, acetonitrile, methanol were studied as the extraction solvents. Among them, methanol showed the largest peak area and was used as the extraction solvent (Figure 2A).

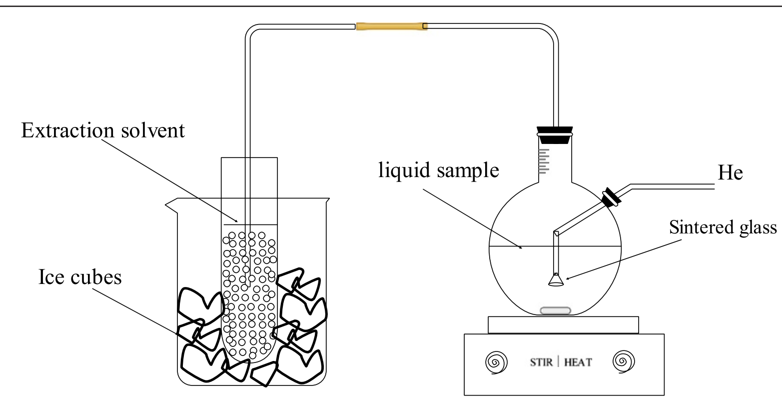

Figure 1 Schematic view of experimental setup.

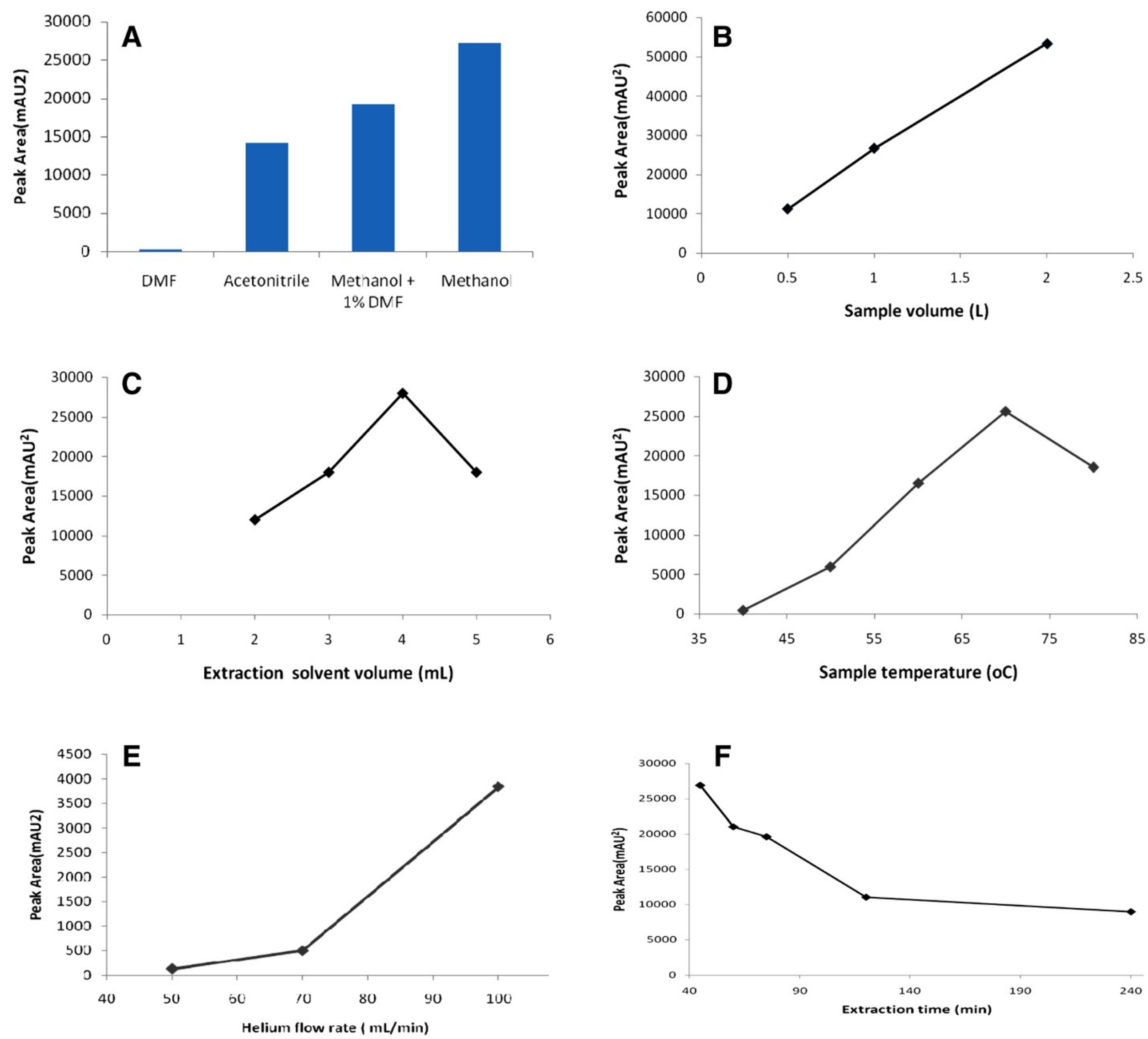

Figure 2 Utilized conditions: furan 0.01 µg mL^{-1}, temperature 40°C, time 45 min, 1,000 rpm, helium flow rate 100 mL min.$^{-1}$. Effect of extraction solvent on the peak area (**A**); effect of the sample volume (**B**); effect of extraction solvent volume (**C**); effect of sample solution temperature, extraction solvent 4 mL methanol (**D**); effect of helium flow rate, extraction solvent 4 mL methanol (**E**); effect of extraction time, extraction solvent 4 mL methanol (**F**).

Optimization of the studied parameters

Sample volume

Sample volume was studied in the range of 0.5 to 2 L. Peak area increased with the increase of the sample volume. The volume of 1 L was chosen as the optimum sample volume. More than 1 L leads to poor purging capacity and precision (Figure 2B).

Volume of the extraction solvent

The volume of the extraction solvent was studied in the range of 2 to 5 mL. Four milliliter was chosen as an optimum volume for the extraction solvent. More than 4 mL leads to a decrease in analytical sensitivity basically due to dilution (Figure 2C).

Extraction temperature

Temperature affects the purging efficicency and transfer rate of analyte from sample solution to gas phase. Purging efficiency increases with increase in the temperature of the sample solution mainly because of increase in the vapor pressure. Extraction temperature was studied in the range of 40°C to 80°C. At temperatures above 80°C,

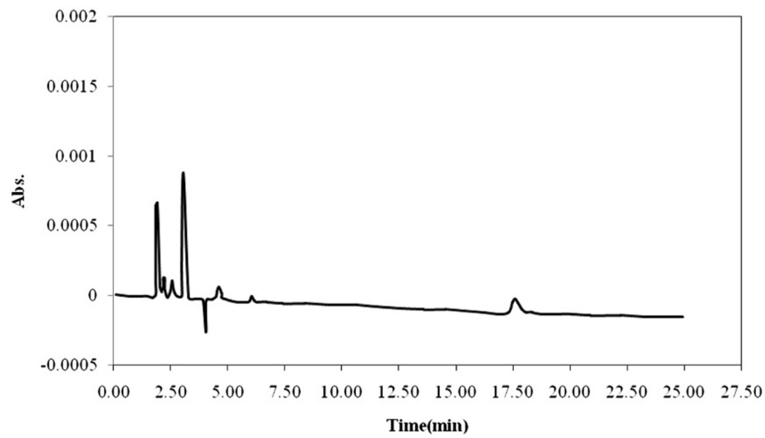

Figure 3 **Chromatogram of 0.001 μg/mL furan standard solution in optimum condition.**

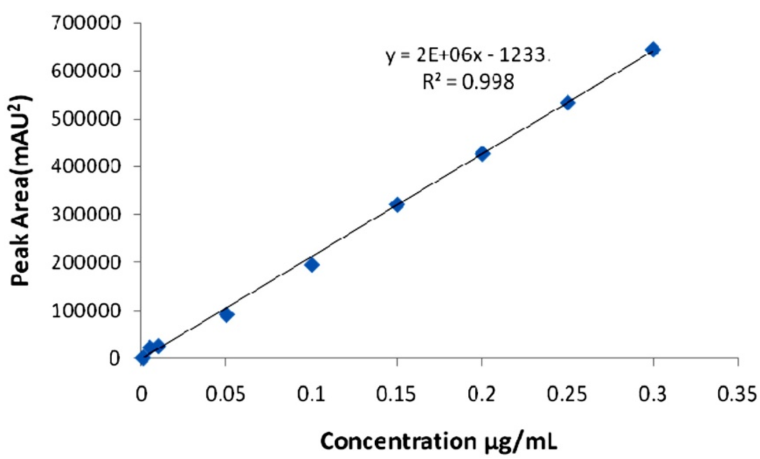

Figure 4 **Calibration curve for furan determination using HPLC.**

helium temperature increases which subsequently decreases the trapping efficiency and precision. The optimum temperature was obtained as 70°C (Figure 2D).

Flow rate

Helium flow rate was studied in the range of 50 to 100 mL/min. By increasing the helium flow rate, volatile species were transferred more rapidly into the trapping solvent, and as a result, the concentration was increased. At higher flow rate of helium, trapping solvent splashed out. The optimum value was obtained as 100 ml/min (Figure 2E).

Extraction time

Furan cannot remain in the trapping solvent for a long time due to its volatility. Extraction time was studied in the range of 45 to 240 min. Forty-five minutes was chosen as the optimum extraction time due to the increase peak area. The peak area was decreased after 45 min (Figure 2F).

Salt effect

The effect of salt was investigated by adding the sodium chloride to the aqueous samples. Peak areas were slightly increased because of salting-out effect. A concentration

Table 1 Analytical characteristics of the method

Characteristic	Value
Slope	2×10^6
Intercept	−1,233
Coefficient of determination (R^2)	0.998
LOD[a] (μg mL^{-1})	0.001
LOQ (μg mL^{-1})	0.005
LOL (μg mL^{-1})	0.300
RSD[b] ($n = 5$)	2.21 (0.005), 2.07 (0.100)

[a]Calculated based on the signal-to-noise ratio of three. [b]Values in parentheses are the furan concentrations (μg/mL) for which the RSD was obtained. LOD, limit of detection; LOQ, limit of quantification; LOL, limit of linearity; RSD, relative standard deviation.

Table 2 Determination of furan in beverage samples

Sample	Concentration (mean ± SD[a], μg/mL)	Added (μg/mL)	Found (mean ± SD[a], μg/mL)	Recovery (%)
Cola (Fanta[b], 1.5 L[g])	0.020 ± 0.002	0.030	0.040 ± 0.003	67
Coffee (Klassno Company Malaysia[c], 18 g[g])	nd[h]	0.030	0.026 ± 0.003	87
Beer (Patrik Company (Iran)[d], 1 L[g])	nd	0.030	0.026 ± 0.002	87
Grape (Takdaneh[e], 1 L[g])	nd	0.030	0.023 ± 0.002	77
Sour cherry (Pakban[f], 1.5 L[g])	nd	0.030	0.025 ± 0.003	83

[a]Standard deviation ($n = 3$); [b]The Coca-Cola Company (Iran); [c] ; [d]; [e]Takdaneh (Iran); [f]Pakban Dairy Company (Alborz, Iran); [g]packaging; [h]not detected. SD, standard deviation.

ration of 0.3% NaCl was selected throughout the experiments.

Analytical performance of the method

According to the above optimum conditions obtained for each factor, the furan chromatogram can be achieved as shown in Figure 3 and in order to evaluate the linearity of the method, a calibration curve over a concentration range of 0.001 to 0.300 μg/ml of furan was obtained (Figure 4). In order to evaluate the linearity of the method, a calibration curve over a concentration range of 0.001 to 0.300 μg/mL of furan was obtained. Linear dynamic range, coefficient of determination, LOD, limit of quantification (LOQ) and relative standard deviation (% RSD) are shown in Table 1. For calculation of LOD, it is considered to be the concentration of analyte that gives a signal that is equal to three times the standard deviation of the blank. This is equivalent to defining the LOD as that concentration at which the signal-to-noise ratio equals 3 at 99% confidence limit:

$$\mathrm{LOD} = \bar{x}_{\mathrm{blank}} \pm 3\sigma_{\mathrm{blank}}$$

Similarly, LOQ in the above equation is defined as $10\sigma_{\mathrm{blank}}$. As shown in Table 1, only small deviations between sequential determinations were found.

Validation of the method and analysis of beverage samples

The proposed methodology was applied for the determination of furan in several beverage samples (cola, coffee, beer, grape, sour cherry) in which, among them, cola was found to have a significant amount of furan (Table 2). The beverage samples were spiked to assess

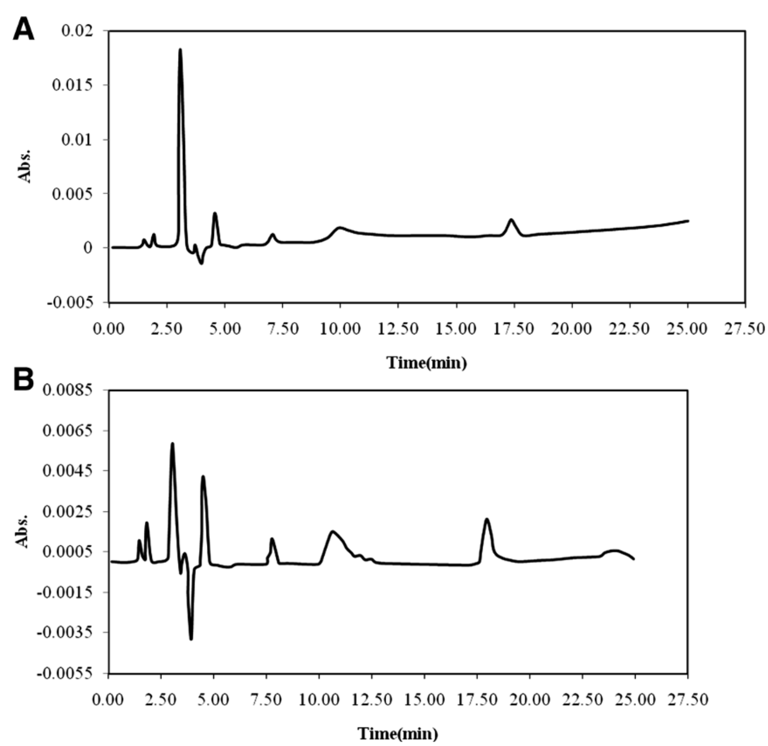

Figure 5 HPLC chromatograms of cola without spike (a) and with spike (b).

the matrix effect. The high recoveries of furan at a level of 0.03 μg/mL showed no matrix interferences. The chromatograms of cola without spiking (a) and with spiking (b) are illustrated in Figure 5.

Conclusions

In this study, a new pre-concentration method combined with HPLC technique was applied for extraction and determination of furan in beverage samples. Some effective parameters such as the extraction solvent, extraction time, sample temperature and volume, helium flow rate and salt effect were investigated and optimized. LOD and RSD were obtained as 0.001 μg/mL and 2.21% ($n = 5$), respectively. The proposed methodology was applied successfully for the determination of furan in cola, coffee, beer, grape and sour cherry which, among them, cola was found to have a significant amount of furan.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HK conceived of the study and participated in coordination. AK participated in experimental design and optimization. EK participated in preconcentration experiments. MT participated in graphical and tables preparation. SK participated in HPLC experiments. MB and HP participated in the statistical analysis of data. All authors read and approved the final manuscript.

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